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To: Dr. R. Ferguson Date: March 7, 1990

From: R. McCuen

Subject: Operations Support Update - First Quarter, 1990

TOBACCO MICROBIOLOGY

Objective: to enumerate and otherwise evaluate the microflora resident in tobacco materials in support of Operations

Status:

These studies, which arise as responses to requests from various sources in Operations, are conducted so as to measure the populations of bacteria, yeast and mold in tobacco and related materials. Prominent among them is the need to determine the effects of storage over time on microbial populations to identify conditions that might permit microbial growth, leading to chemical changes that affect subjectives. Studies conducted during 1989, and those presently in action, are listed below with requestors identified. (Completed studies are identified with an asterisk.)

Storage Studies

A. Hogshead/Box/Bale Storage Study (F. Jones)

Over three-year periods, monitor the microbial populations of bright and burley strip stored in hogsheads, boxes or bales. The major finding has been that both tobaccos stored in boxes tend to manifest mold growth more than those stored in hogsheads or bales, but bacterial and yeast growth is minor. Ongoing study.

B. "Famous" Blend Storage Study (L. Jennings)

The microbial populations of a modified Chesterfield blend for export ("Famous") were monitored at monthly intervals during six months of storage under jungle, desert and ambient conditions. Jungle storage was terminated at eight weeks owing to excessive mold growth, but storage under desert and ambient conditions produced no notable population changes. Assays of chemical constituents are incomplete at this writing.

C.* Bulk Tobacco Handling Storage Study ("Rolling Silo") (R. Bowman)

RLB, RLTC, RCB and MT held in a mobile silo at various OV values and time periods (up to 72 hr) were monitored for population changes. Such storage was uneventful, with populations similar to those found in materials stored in hogsheads.

D.* Post-ART Process Filler Storage Study (J. Baggett, B. Taylor)

ART filler, held at 35°C/80% RH and at 25°C/60% RH for periods up to 12 weeks, manifested no significant changes in bacterial or mold populations. A similar result was obtained using ART filler (stored for up to 7 days in the cold) at 35% and 12% OV. Both sets of ART fillers contained elevated yeast populations at time zero, but this has not been observed in subsequent ART studies.

E.* ART Cigarette Storage Study (R. Carchman, W. Hempfling)

Cigarettes containing cased and uncased ART tobacco, and unextracted tobacco, were stored in open trays under desert and jungle conditions for periods of up to eight weeks. ART-extracted and unextracted cigarettes were stored in separate jungle rooms to avoid nicotine transfer. No notable changes of bacterial or yeast populations occurred. Some excitement arose due to extensive growth of mold on the ends of the ART models by six weeks, but subsequent work showed that airborne fungal contamination was the likely cause of that bloom. Subsequently, placing unextracted cigarettes in the contaminated jungle room also resulted in mold growth on the cigarette ends.

F.* Burley (20% Casing) Storage Study (M. Tallman)

Burley tobacco cased at a 20% level contained unchanged populations of bacteria, yeast and mold after storage at room temperature for 48 hr.

G.* Pre-DIET Process Storage Study (K. Houghton)

Bright strip (18% OV) and cut bright filler (22% OV) prepared for DIET processing was stored in PM-80 boxes for up to 7 days. Mold populations were slightly increased by Day 4, but bacterial populations did not change over the entire period.

H. Alternate Humectant Storage Study (R. McCuen)

Control (PEG+G) and test (Isosweet) sheets of RL150B, RLTC and RCB contained similar microbial populations at time zero. This is an ongoing warehouse storage study.

I. PM-80 Box/Liner Storage Study (focus on mold) (F. Jones)

Bright and burley strip, stored in PM-80 boxes for up to three years, will be monitored to determine if two different kinds of box liners result in different rates of mold growth. Time-zero samples of bright contained mold and yeast populations above specifications, but burley contained lower populations. This is an ongoing storage study.

J. Ivory Coast-Senegal Blend Storage Study (B. Semp)

Microbial populations of a tobacco blend intended for the Ivory Coast and Senegal were measured. Similar measurements will be performed on samples of that material by FTR when the tobacco has reached its destination.

K.* Foam-Bonded Ends Solution Storage Study (S. Wrenn)

The susceptibility to microbial growth of an aqueous solution containing sodium carboxymethylcellulose and licorice (used for foam-bonding cigarette ends) was determined at room temperature and in the cold. After one day at room temperature increased yeast populations were detected, while storage in the cold prevented such growth. A recommendation was made to store the solution in the cold.

Other Studies

L. Dry Flavor Study (K. Houghton)

Multiple collections of dry flavor components and mixes have been made at the Manufacturing Center, Park 500, Twentieth Street and Stockton Street facilities. Populations of bacteria, yeast and mold were measured in each component and mix. From all facilities, neither bacteria, yeast nor mold were detected in Shiloh, Police, Candy, Candy M-40, or Petreo. DAP and urea from P-500 were also free of detectable microorganisms. Cochise consistently contained about 10^5 bacteria and 10^4 mold per g, while Jono contained about 10^4 bacteria per g, but was practically free of mold. When properly sampled, burley spray was free of bacteria or mold. At P 500, Cochise/urea contained about 10^3 bacteria per g and Jono/DAP contained about 10^2 bacteria per g. This was true even after 36 hr following formulation. Information about mold in those mixes is incomplete, as is all information about Dry Export Mix from the Twentieth Street facility. This study is to end in January 1990.

M.* Centrifuge Sludge Study (T. Bullock)

Because the re-addition of centrifuge sludge to the RL sheet at Park 500 is under consideration, the microbial populations of sludge from each centrifuge (Line 3) were measured. Only typical tobacco bacteria (bacilli) were found (about 10^8 per g dry weight), and the bulk of them were present as endospores. Preparation of heat-dried pellets from that sludge contained only about 1% of the original bacterial populations in fresh sludge. Neither mold nor yeast was detected in centrifuge sludge.

N.* SEL Nebulization Study (T. Bullock)

Aerosols produced by nebulization from SEL containing bacteria contained less than 10% of the starting population density in the SEL. A quantity of SEL was rendered sterile by sequential membrane filtration and supplied to Dr. R. Carchman for further studies.

O.* ART Water Scrubber Liquor Study (D. Howe)

Water scrubber liquor from the ART Pilot Plant was examined for bacteria, yeast and mold and was found to be free of microorganisms.

P. **Nicotine Degradation in P500 Waste-Water Treatment (WT) System**
(C. Ellis)

Objective: to investigate nicotine degradation in samples from WT system (primary and aeration basins only) for estimation of nicotine-removal capability, and for potential linkage to RT (water column) process.

Status:

The project was initiated 9 January 1990. The levels of nicotine in waste water entering and exiting the primary basin (PB) are 30 - 40 ppm, but nicotine is not detectable in the aeration basin (AB). Rates of removal of added nicotine in PB water are slow, but AB water eliminates nicotine at about 45 ppm per hr (72°F). The site of major nicotine elimination is hence the aeration basin. A method for detection and enumeration of nicotine-metabolizing bacteria has been developed (at least eight different types of such bacteria are present).

Plans:

1. Continue on-going storage studies (A., H. and I., above).

2. Complete "Famous" study and Dry Flavor study (B. and J., above).

3. In study "P" the following will be done:

   a. Measure instantaneous rates of nicotine degradation in samples from aeration basins as a function of temperature (complete January 1990).

   b. Measure and identify populations of bacteria active in nicotine dissimilation (complete March 1990).

   c. Identify conditions (laboratory) necessary for complete removal of nicotine from ART water column effluent, using starting cultures from WT system (complete April 1990).

   d. Identify intermediate and end products of nicotine degradation (complete June 1990).

4. Continue to respond to requests for studies in support of Operations.

ALTERNATE PRESERVATIVES PROGRAM

Objective: to identify tobacco-identical or otherwise "natural" compounds to replace or supplement propylparaben (PPB) so as to minimize or eliminate microbial activity in changing chemical composition of SEL during processing.

Status:

To date (January 1990), of some 102 compounds tested, 12 compounds other than PPB have been identified as preventing growth of bacilli at

levels of 250 ppm or less (Phase I agar-inclusion and shake-flask assays). Five of these compounds were effective in preventing growth in SEL (laboratory scale), but two of these were subjectively unacceptable (decanoic acid and β-cyclocitrylidene acetic acid); citronellol, carvacrol and thymol have not been subjectively tested. Seven compounds have not been tested in SEL (trans-cinnamaldehyde, nonanoic acid, undecylenic acid, lauric acid, decanol, dodecanol and σ-dodecalactone).

Plans:

Work was suspended pending completion of study of nicotine degradation in P500 waste-water treatment system. Resume in 2Q 1990, with recommendations for testing at P500 available 4Q 1990 (preservatives selected that are both efficacious at low levels and subjectively acceptable).

ALTERNATE HUMECTANT PROGRAM

Objective: to produce an acceptable full-flavored cigarette which is PG/G-free.

Status:

1. All control and PG/G-free feedstocks (prepared in the production facilities - RL, RCB, ET and ES), containing various amounts of Isosweet with K-pp where appropriate, were analyzed again after storage for subjective, microbiology and chemical composition. Results showed that all feedstocks were equivocal to the controls except in the amount of PG/G and sugars (as expected).

2. Time zero survivabilities for the RL and RCB sheets were completed in a satisfactory manner.

3. Nine different PG/G-free flavor and casing models have been made by Flavor Development personnel. The only model to give reasonable subjectives was one in which the PG/G was replaced with Isosweet.

4. Three small-scale Isosweet models (with 100%, 85% and 50% of the PG/G replaced with the sugars) and a control were made by the Flavor Development Division personnel. All three of the test cigarettes were not "in spec" as far as the analyticals were concerned and they were not subjectively acceptable.

5. A large-scale making run was conducted in mid-October in which the PG/G was replaced with Isosweet on a 1:1 weight basis. Two kinds of test cigarettes were made (0 and 10% dilution) and suitable controls. Survivabilities were done across the maker as well as cigarette analyticals and physicals. All test cigarettes were judged not different from the controls except in the analyticals (where expected) AND in subjectives.

Plans:

Continue to develop PG/G-free casings and flavors for use on small-scale produced cigarettes until subjective parity is obtained with a suitable control. Produce large-scale cigarettes based on the above model and determine survivabilities in the Primary and Make-Pack areas of the Semi-works. If the subjectives of these cigarettes are equivalent to the control, 2-POLs will be initiated about mid-year to qualify these materials.

/mps

cc: Dr. C. Ellis

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